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THE ANTIBACTERIAL EFFECT OF POMEGRANATE PEEL AND GUAVA LEAVE EXTRACT MOUTHWASH ON ORAL STREPTOCOCCUS MUTANS IN SCHOLAR STUDENTS: A CONTROLLED CLINICAL STUDY

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ABSTRACT

Objective: Pomegranate peel extract (PPE) and guava leave extract (GLE) were used for centuries and their therapeutic abilities were reported. Hence, this study was conducted to evaluate and compare the antimicrobial effect of mouthwashes prepared from pomegranate peel and guava leave extract on oral Streptococcus mutans (S. mutans) count in healthy subjects. **Subject and methods:** A total of one hundred healthy children aged between 6-12 years were enrolled in this study and equally divided into five equal groups (n=20) according to mouth rinse type used in this study. Group 1 used 2% chlorhexidine (CHX) mouthwash; group 2 used 10 % PPE mouthwash; group 3 used 15 % PPE mouthwash; group 4 used 10 % GLE mouthwash; while group 5 was use15 % GLE mouthwash. Samples of Saliva were collected after mouth rinsing at two-time intervals; immediately after mouth rinsing and 2 hours after rinsing. Mitis Salivarius agar medium was used to determine S. mutans count. **Results:** The results revealed that S. mutans count recorded a marked significant reduction in all studied groups immediately after mouth rinsing, then S. mutans count was increased significantly in all groups after 2 hrs. The lowest S. mutans count was recorded with the CHX group followed by15 % and 10% PPE groups respectively. While the lowest performance was observed in GLE 15% and 10 % groups respectively. **Conclusions:** The use of PPE mouthwash has a higher antimicrobial effect when compared to GLE in both concentrations.

KEYWORDS: Pomegranate Peel, Guava Leave Extract, Mouthwash, Streptococcus Mutans

INTRODUCTION

Tooth caries is one of common chronic diseases between children and young adults' population [(1)]. Streptococcus mutans is a gram-positive anaerobic bacterium that is found usually in the oral cavity and is stated as the main bacteria which begin the carious lesion [(2)]. So, effective mouthwash should have the capacity to alter the oral microbiota via selectively eliminating the harmful bacteria without adversely assuming the commensals microorganisms [(3)].

Chlorhexidine is the gold standard mouthwash with considerable anti-plaque action and antimicrobial properties but it can cause tooth and tongue staining, taste alteration, and raise the opportunity for calculus development[(4)]. Consequently, a modern and safe antibacterial mouthwash development

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is substantially recommended particularly in children [4,5].

Throughout the ages; nature was a qualified source for herbal materials of antimicrobials potential through pathogenic microorganisms due to their chemical counterparts [4,6]. The pomegranate fruits; flesh, and peel have been extensively utilized in the traditional medicine as a therapy for several kinds of diseases [7]. Pomegranate has several antioxidant activities and the revealed clinical trials emphasis that this antioxidant property provides pomegranate with significant strength to aggression oral pathogens [8].

Guava leaves have a large set of bioactive compounds like tannins, triterpenes, essential oils, carotenoids, saponin, vitamins (C&A), alkaloids, and glycosides, that record worthy medicinal goods throughout the history of humanity[9]. One of these active ingredient "flavonoids" has an antibacterial influence plus "quercetin" that has the anti-oxidant effect that improves the function of the immune system [10].

So, this study was aimed to estimate the effect of pomegranate peel and guava leaves extract in two concentrations as mouth rinses on S. mutans count in saliva and compare it with the standard chlorhexidine (CHX) mouth rinse in a group of children.

SUBJECT AND METHODS

The current study was conducted on a total of one-hundred healthy school-aged children ranged between 6-12 years after approval of Ethical Committee of Faculty of Dental Medicine, Boys, Cairo, Al-Azhar University (EC Ref No.: 120192/3/21). Subject selection was designed on the absence of any systemic disease, orthodontic appliances, or antibiotic drug medication for at least 15 days before starting [6,11]. Written informed consent was taken from the parents of each involved child. Subjects were equally divided into five equal groups (n=20) according to mouth rinse type: group 1 received 2% CHX mouth rinse; group 2 received 10 % pomegrenate peel extract (PPE) mouth rinse; group 3 received 15 % PPE mouth rinse; group 4 received 10 % Guava leave extracts (GLE) mouth rinse; while group 5 was received 15 % GLE mouth rinse.

Preparation of mouth rinses

Pomegranate peel and Guava leave extracts were used to prepare the mouthwashes based on Minimum inhibitory concentration (MIC) using distilled water. Although no sweeteners were added, the taste was found to be acceptable. Chlorhexidine (2%) (Gluco-Chex 2%, Erkamed Co.) was used as a control. Volume and time of wash were standardized to be 15 ml for 2 minutes/wash. The extracts of pomegranate and guava leaves were purchased from (scale chemicals company). The extract was stored in a dark bottle and kept in refrigerator[6,11]. Chlorhexidine (2%) was used as control. Chlorhexidine 2% mouth wash was used as a control.

Saliva sample collection:

Samples of saliva were collected before mouth rinsing from each enrolled subject. Then, each enrolled child (both sexes) in each group was instructed to rinse the mouth with 15 ml for 2 minutes. After mouth rinsing, samples of saliva were collected from each enrolled subject at two-time intervals; immediately after mouth rinsing and 2 hours after rinsing. Each subject was allowed to spit in a sterile wide-necked container under complete aseptic conditions. Samples were delivered to the microbiological lab at Microbiology and immunology Department, Minia university for culturing on selective media of Mitis Salivarius Bacitracin (MSB) agar medium to determine S. mutans count [6,11].

Statistical analysis

The obtained data were tabulated and statistically analyzed using SPSS version 15.0. ANOVA test was used to compare among the five groups

for normally distributed quantitative variables and Post Hoc test (Tukey) for pairwise comparisons. ANOVA with repeated measures was used for normally distributed quantitative variables, to compare between more than two periods and Post Hoc test (Bonferroni adjusted) for pairwise comparisons.

RESULTS

The comparison among the different studied groups according to S. mutans count is presented in (Table 1). Our results revealed that S. mutans count registered a marked significant ($p<0.01$) reduction in all groups at once after treatment and thereafter it raised significantly in all groups after 2 hrs. No significant differences were showed between the five groups at the pre-rinse test but immediately after mouth rinsing, a significant reduction in the S. mutans count was recorded in chlorohexidine and 15% PPE groups. The lowest rendering was noticed in the 10% GLE group. After 2 hours, the CHX group recorded the lowest bacterial count followed by a significant difference by 15% PPE and 10% PPE groups, and the lowest performance was reported in 15% GLE and 10 % GLE groups respectively.

TABLE (1): Comparison between the different studied groups according to *S*. mutans in children.

***S.* mutans count ($\times10^3$)**	**G1 2% CHX**	**G2 15% PPE**	**G3 10% PPE**	**G4 15% GLE**	**G5 10% GLE**
Pre-rinse	$186.5^{A} \pm 49.1$	$166.1^{A} \pm 42.7$	$180.6^{A} \pm 48.6$	$184.9^{A} \pm 46.1$	$195.1^{A} \pm 42.2$
Immediate	$36.4^{Bc} \pm 11.2$	$43.6^{Bc} \pm 15.4$	$62.4^{Bb} \pm 16.7$	$66.88^{Bb} \pm 15.9$	$79.95^{Ba} \pm 17.2$
After 2hrs.	$40.4^{Cc} \pm 12.6$	$48.0^{Cc} \pm 17.0$	$66.2^{Cb} \pm 23.1$	$80.2^{Ca} \pm 19.0$	$88.0^{Ca} \pm 18.9$

Means in the same column (between periods) with different capital superscript letters are significantly different
Means in the same row (between groups) with different small superscript letters are significantly different

DISCUSSION

Tooth decay is a chronic contagious disease that resulted because of bacterial colonization on hard tooth structures and although the great efforts exerted worldwide to reduction its incidence, its prevalence is still rise [11,12]. Mouth rises are considered a remarkable agent to minimize oral bacterial colonization along with the frequent mechanical methods [13].

As Adair reported [14], mouth rinses should be described only for children who have established adequate mastery monitoring of their swallowing reflex. So, in this study, the choice children were aged among 6-12 years as they can facilely rinse their mouths without swallowing the rinsing solutions to avert deglutition reflex's [11].

In the current study, S. mutans was selected as a tested bacterium for the comparison of the antimicrobial activity among the tested mouth rinses. This is due to S. mutans is the bacterial species that responsible for the initiation of caries[2]. The MSB media was chosen in this study for isolation and counting of the S. mutans colonies due to it is the selective media for such colony's growth and inhibition of other microorganisms [15].

A 2% CHX mouth rinse was utilized as an active monitoring in the present study. As CHX is the ultimate effective antimicrobial agent until now and the ultimate popular, as well as it is qualified as the gold standard between the marketed mouth rinses [4,5]. While, because of its common disadvantages, there is a necessity to subedit other mouth rinses

with safety measures particularly for children[4,5]. In our study, PPE and GLE were chosen as tested mouth rinses due to their capacity to inhibit microbial colonization and prevent its adhesion to the hard tooth surfaces [4,16, 17].

This study revealed that the difference in S. *mutans* count before Using the tested mouth rinses was statistically non-significant between all tested groups, so this referred to standardization for bacterial counts per the studied groups [18].

Our study showed that, both PPE and GLE presented a significant decrease in S. mutans count at once after utilization. This could be regarding to the prevalence of polyphenolic flavonoids as an active ingredient in both extracts [6,11]. Additionally, the result of the present study showed the higher concentration in both studied groups (PPE and GLE) has the higher antibacterial influence. That could know by the raise the amount of their active ingredient with an excess of concentration i.e., their influence is dose-dependent [11,19].

On the other hand, the finding of our study revealed that subjects who rinsed with PPE experienced a higher reduction in *S*. mutans count when compared to subjects who rinsed their mouths with GLE at both studied concentrations. This may be due to pomegranate peels has a higher amount of antioxidant and flavonoids when compared to guava leaves [20].

Furthermore, the finding of the current study reported that CHX mouth rinse has a significantly higher influence concerning S. mutans count reduction. This is due to CHX is a cationic molecule that has the capacity to binds to the negatively charged bacterial membrane and change its permeability and hence kill it [21].

While, the significant raise in S. mutans counts after 2 hours of mouth rinsing between the all-tested groups with significant difference among CHX and other mouth rinses in our study, may be due to the rapid drop of salivary pH or to the short duration of action of the utilized mouth rinses [22].

CONCLUSION

Based on the finding of the present study we can imply that PEE and GLE have an antimicrobial influence however, PEE has the marked antibacterial efficiency regarding *S*. mutans species.

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